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Low-Dose Ionizing Radiation Modulates the Expression of Proangiogenic Genes in Critical Limb Ischemia Patients: Preliminary Results

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Objective: Low-dose ionizing radiation (LDIR), namely, 0.3 Gy, delivered during 4 consecutive days, has been reported to stimulate angiogenesis and arteriogenesis in a preclinical model of hindlimb ischemia. Here we performed a single-center, investigator-blinded, randomized, sham-controlled clinical trial to evaluate the effects of LDIR exposure in the expression of proangiogenic genes in endothelial cells isolated from muscles of patients with critical limb ischemia.

Methods: "Non-option" critical limb ischemia patients were randomized to receive 0.3 Gy or 0.0 Gy delivered for 4 consecutive days. By use of a PALM MicroBeam Laser Microdissection System (Zeiss, Oberkochen, Germany), the endothelial cells were exclusively isolated from ischemic gastrocnemius muscle samples collected at amputation, and the expression of proangiogenic genes was analyzed. In addition, LDIR stimulation of angiogenesis and arteriogenesis was assessed through measurement of the capillary vessel density using CD31 immunohistochemistry and eosin counterstain and the collateral vessel density by lower limb digital subtraction angiography. Finally, the safety and feasibility of LDIR exposure and the potential therapeutic benefits were documented.

Results: Between December 2015 and February 2017, there were 16 patients aged between 48 and 78 years with intractable critical limb ischemia who were enrolled in the study. Four patients withdrew or were excluded. Twelve patients corresponding to 13 limbs were considered for analysis. Five limbs were randomized to receive LDIR, and the other eight underwent the sham protocol. Baseline patient and limb demographic and clinical characteristics were similar by study arm. Regarding the primary end point, transcript levels for hepatocyte growth factor, angiopoietin 2, and vascular endothelial growth factor receptor 2 trended toward upregulation in endothelial cells isolated from irradiated samples, exclusively in limbs exposed to LDIR. A trend toward a capillary vessel density increase and a significant collateral vessel density increase in irradiated limbs was documented compared with the sham-irradiated limbs ($P = .032$). Effects of LDIR on surrogate clinical end points of ischemia were similar in both groups.

Conclusions: These preliminary data show a trend toward an increased expression of proangiogenic factors in irradiated limbs, and simultaneously LDIR is consistently associated with an increase in capillary density and a statistically significant increase in collateral vessel density after angiographic analysis. As expected, because of the adverse selection of patients with advanced disease, LDIR therapy was not expected to reduce 1-month amputation rates or other clinical end points of ischemia. Increasing the size of the study population, selecting patients in less advanced stages, and extending the follow-up period will allow us to adjust the indications for use of LDIR in patients with peripheral artery disease to maximize its therapeutic potential.

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